Preparative Fractionation by Frontal Countercurrent Distribution

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Summary

The frontal (multiple-input) approach is applied to countercurrent distribution. Equations are derived for calculating the number of tubes required for a given separation or for calculating the amount of solute emerging at the intersection of two solute profiles. In both cases ideal behavior is assumed. The applicability of these equations to preparative fractionations is evaluated and the utility of the frontal technique demonstrated.

Several approaches exist for increasing the amount of material that can be fractionated in preparative countercurrent distribution (CCD) and that maintain output profiles similar to those predicted by the use of simple mathematical relationships. This is necessary so that the possibility of fractionating solutes of given partition coefficients can be ascertained and the effect of varying experimental conditions evaluated. One commonly used approach involves introduction of solute into a number of early CCD tubes and use of the single withdrawal technique. An alternate approach was suggested in a recent paper (*1*) from this laboratory. It was demonstrated that when solute increments are added to the zeroth tube of a countercurrent distributor for a large number of inputs, a "frontal" output curve results (Fig. 1). It was shown that under ideal conditions such frontal curves could be described as the integral of the Gaussian function. Ideality means here that partition coefficients and solvent volumes are constant throughout the distribution and that all of the upper phase but no lower is transferred. Equations were derived for cal-

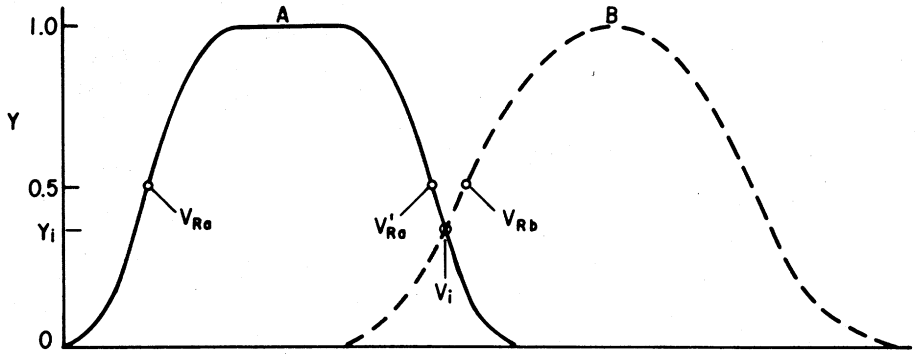


FIG. 1. Frontal CCD output profile.

culating the total volume, V, of upper phase transferred to any value of Y on an output profile and for the number of inputs (N_F) required to just make a frontal output. N_F increases as the number of tubes increases and also as K_D decreases.

$$V = V_R + t\sqrt{V_L V_R / K_D}^* \quad (1)$$

$$N_F = V_F/V_T;\ V_F = 4.66\sqrt{V_L V_R/K_D} = 4.66\sqrt{[V_L p(V_U + V_L/K_D)]/K_D} \quad (2)$$

Partition coefficients can be determined from equilibrium experiments. The Bush and Densen equation ($V_L/V_U = \sqrt{K_{D_1}K_{D_2}}$) gives, under certain conditions, the optimum solvent ratio for the separation of two components (*2*). If this ratio is accepted as the appropriate one, an expression for calculating the number of plates required for a given separation can be derived. Consider the ideal frontal curves in Fig. 1 where B reaches a plateau and A overlaps B at (V_i, Y_i). Any value of Y_i can be chosen and the corresponding value of t obtained. There is a point ($V'_{R,a}$) on the trailing edge of A around which that edge is symmetrical. The volume transferred to that point is $V_{R,a} + 0.5V_{F,a} + (V_{F,b} - V_{F,a} + 0.5V_{F,a})$. Then from the properties of a Gaussian curve:

$$V_i = V'_{R,a} + t_{i,a}\sqrt{V_L V_{R,a}/K_{D,a}} \quad (3)$$

and for curve B:

$$V_i = V_{R,b} + t_{i,b}\sqrt{V_L V_{R,b}/K_{D,b}} \quad (4)$$

* Symbols defined at end of paper.

The term $(V_{F,b} - V_{F,a})$ arises from the fact that more additions of A have been added than were necessary to just make a frontal (see Eq. 2). It represents the length of the plateau. By equating (3) and (4), substituting for V_F and V_R, and rearranging, Eq. (5) is obtained.

$$\sqrt{p} = \frac{(4.66 - t_{i,b})K_{D,a}\sqrt{1 + K_{D,b}(V_U/V_L)} + t_{i,a}K_{D,b}\sqrt{1 + K_{D,a}(V_U/V_L)}}{K_{D,a} - K_{D,b}} \quad (5)$$

Alternatively, Eqs. (3) and (4) may be solved for t and the Y value at the intersection point found. Since $t_{i,b} = -t_{i,a}$

$$t_{i,b} = (V_{R,a} - V_{R,b} + V_{F,b})/(\sqrt{V_L V_{R,b}/K_{D,b}} + \sqrt{V_L V_{R,a}/K_{D,a}}) \quad (6)$$

The purpose of this research is to apply the frontal approach to the countercurrent separation of complex mixtures and to investigate the applicability of equations derived above.

EXPERIMENTAL

Materials and Methods

Lard methyl esters were prepared using sodium methylate as catalyst (*3*). The composition of the esters, as well as the composition of solutes from partition coefficient experiments using hexane–acetonitrile as solvents, were determined by GLC (*4*). These esters were considered for CCD simulation: stearate (12.6%, K_D 13); oleate (43.9%, K_D 7); palmitate (23.9%, K_D 8); myristate (1.6%, K_D 5); palmitoleate (3.1%, K_D 4); linoleate (10.6%, K_D 4).

Refined *Vernonia anthelmintica* seed oil, low in free fatty acid (*5*), was assayed by preparative thin-layer techniques (*6*) using solvent systems which have been described previously (*7*). These fractions were compared with known compounds where possible on TLC and also converted to methyl esters and analyzed by GLC (*8*). The composition was as follows: 62% of the triglyceride of 12,13-epoxy-9-octadecenoic (vernolic) acid, 22% divernoloyl-acyl-triglyceride, 10% monovernoloyl-di-acyl-triglyceride, 1% normal triglycerides and nonpolar unsaponifiable material, and 5% free acids, partial glycerides, and other unsaponifiables. Partition coefficients in hexane-acetonitrile were determined at 25°C. The aforementioned methods were employed to analyze the solutes contained in the upper and lower phases. For simulation of CCD, three components were considered: trivernolin (K_D

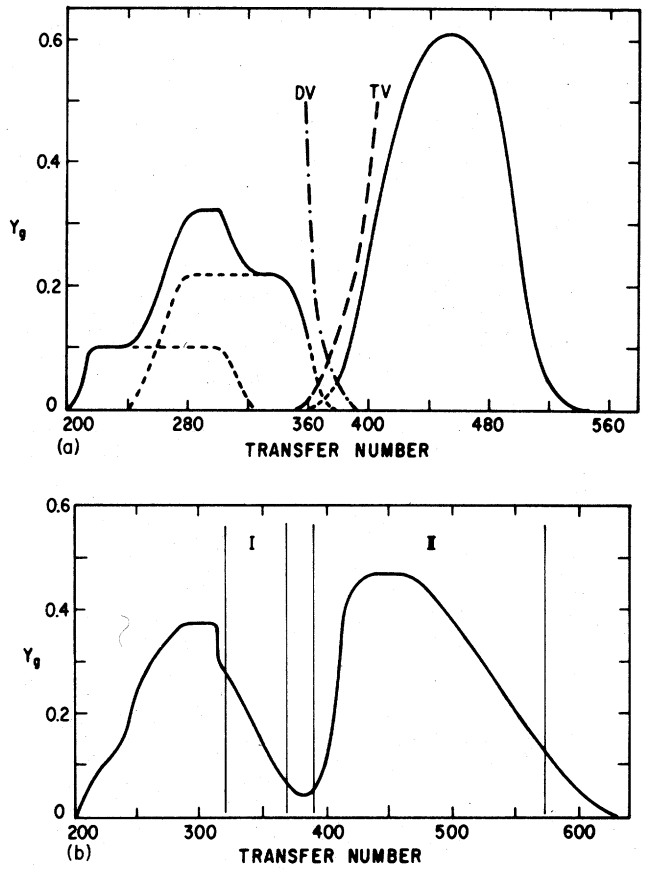
1.9), divernoloyl-triglyceride (K_D 6.4), and less polar triglycerides (K_D 33).

Frontal countercurrent fractionations were performed as described previously (*1*). Counter double current distribution (CDCD) data were obtained by computer simulation (*9*). Steady-state operations were simulated for a 100-tube distributor with solute being fed into Tube 50.

RESULTS AND DISCUSSION

Ideal conditions are seldom encountered in experiments. Volume reorganizations, incomplete mobile-phase transfer, stationary-phase transfer, and other effects often influence the positions and shapes of frontal output profiles. In order to test the equations derived here for ideal conditions, the countercurrent process was simulated by computer (*10*). No predetermined mathematical description of the output profiles was assumed in the simulation. The system consisted of 20 ml hexane, 39.2 ml acetonitrile, and 1 g each trivernolin (TV) and divernoloyl-acyl-triglyceride (DV). The number of plates required to resolve these solutes was calculated by Eq. (5) for $Y_i = 0.2$ and $Y_i = 0.7$. The values of p found were 137 and 61, respectively. When these p values were used in the simulation, the required intersection points were obtained. Thus, the approach leading to Eq. (5) was verified. The results also demonstrate that good resolution of TV and DV would be obtained with the 200 tube appartus at our disposal. In fact, Eq. (6) predicted that $Y_i = 0.08$ using the solvent volumes given above. The intersecting portion of the profiles is shown in Fig. 2a.

Idealized frontal output profiles were plotted for the Vernonia oil and lard methyl esters using Eq. (1). To obtain curves that are more meaningful in terms of quantities fractionated, each Y was multiplied by the fraction of that component in the mixture. These profiles are shown in Figs. 2a and 3a. N_F for the mixtures was taken as N_F for the component of greatest V_R in the mixture. Additional inputs would lengthen all of the plateaus. Figures 2b and 3b depict the experimental output profiles. Although the Bush and Densen equation predicted that a solvent ratio of 0.29 (upper/lower) would give optimum separation of the divernoloyl triglyceride and trivernolin regions, the ratio 0.51 was chosen because emulsion problems were encountered at oil feed levels above 0.5 g at the former ratio. Thus, less oil could be fractionated and less of the products recovered even though overlapping was reduced.



. Vernonia oil fractionation. Predicted using Eqs. (1) and (2). $V_U = 20$ ml, $V_L = 39.2$ ml, $p = 200$, 95 inputs—1.00 g each. (—) Total weight curve. DV and TV are portions of normalized profiles.

FIG. 2b. Vernonia oil fractionation. Experimental profile. $V_U = 20.0$, $V_L = 39.2$ ml, $p = 200$, 100 inputs—1.07 g each.

Intersection points do not occur at the same transfer number in the Y and Y_g profiles. On Fig. 2a, for example, curves DV–TV intersect at 373 transfers, while the weighted profiles intersect at 369 transfers. An illustration with Gaussian curves is useful at this point. Curves A and B in Fig. 4 are equal in height and intersect at $V = 68$ ml. Curves C and D were drawn through points found by multiplying points on Curve B by 0.5 and 0.1, respectively. The intersection volume is higher as the curve height decreases. Since t increases when

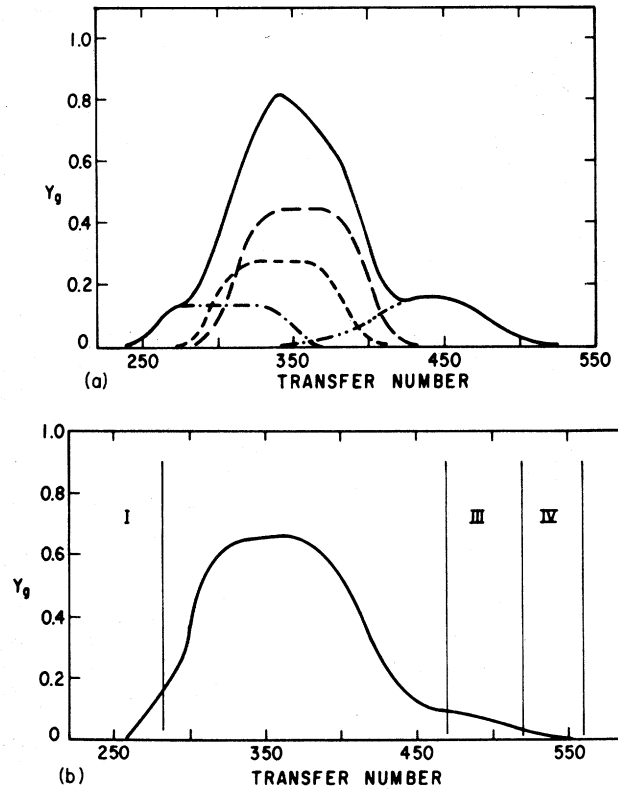


FIG. 3a. Lard methyl ester fractionation. Predicted using Eqs. (1) and (2). $V_U = 10.0$, $V_L = 39.2$, $p = 200$, 92 inputs—1 g each. (—) Total weight curve.

FIG. 3b. Lard methyl ester fractionation. Experimental profile. $V_U = 10.0$ ml, $V_L = 39.2$ ml, $p = 200$, 100 inputs—1.12 g each.

$(V - V_R)$ increases $[t = (V - V_R)/\sigma]$ (*1*), from probability tables we see that Y_A, the fraction of area under Curve A to the intersection point also increases. Hence, in a weighted frontalgram, the intersection shifts away from the larger curve. Considering this, Eq. (6) is valid only for Y plots, whereas Eq. (5) is applicable to both Y and Y_g plots. In the former, the "t's" would be equal but opposite in sign. In the latter, the weight in grams of each component emerging at the intersection point is set equal and the appropriate "t's" obtained for the calculated Y values, paying careful attention to sign.

The illustration (Fig. 4) shows also that the term "resolution" (*11*)

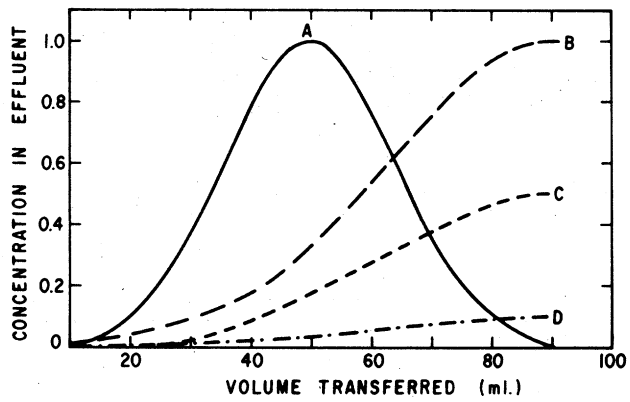


FIG. 4. Gaussian profiles. $K_{D_A} = 1$, $K_{D_B} = 0.5$, $p = 10$, $V_U = 1$ ml, $V_L = 4$ ml. Curves A and B calculated using Eq. (1).

is ambiguous for overlapping Gaussian CCD peaks. Since $R_s = (V_{R,b} - V_{R,a})/4\sigma$ and $\sigma = (V_L V_R/K_D)^{1/2}$, R_s will be invariant for a particular pair of compounds. But it was just demonstrated that the intersection point and the area of A under B varied as the peak heights changed. Thus, the resolution concept seems to have little utility for CCD.

Influences on the shapes of frontal output profiles of model compounds have been discussed elsewhere. It was demonstrated that phase equilibria data, together with computer simulation, were required to make accurate predictions of output profiles in real experiments. Nevertheless, the equations given here are useful for quickly determining whether a given separation should be attempted. By calculating V_R, V_F, and Y_i for components of a mixture, output profiles can be quickly sketched. This information also provides a starting point from which to begin computer simulation when more precise evaluation of parameters is required.

Results from the frontal separations are given in Tables 1 and 2. Also shown are the yields obtained from simulated elution CCD and continuous feed CDCD. These simulations assume ideal conditions and usually represent maximum recoveries. Considering this, both frontal runs yielded significantly more product, at comparable purity, than the elution CCD mode. It seemed reasonable to compare frontal CCD and CDCD yields at the same number of transfers (CDCD inputs), although at steady state more product would emerge with

TABLE 1

Comparison of Extraction Methods: Vernonia Oil

Method	Quantity fed		Recovered in effluent		Purity	
	TV[d] (g)	DV[e] (g)	TV (g)	DV (g)	TV (%)	DV (%)
Elution[a]	13	4	13	4	95+	95+
Frontal[b]	66.3	23.5	63.5[f]	9.6[g]	95+	86
CDCD[c]	102	36	69	26	95+	62

[a] Calc using Gaussian approx (*1*). 20 g oil treated as if it could all be placed in Tube 0. V_U = 20 ml, V_L = 39.2 ml.

[b] Experimental results.

[c] Computer simulation. V_U = 15 ml, V_L = 50 ml. Data shown for 575 transfers. Steady state reached at 311 (0.285 g fed per input).

[d] Trivernolin.

[e] Divernoloyl-acyl-triglyceride.

[f] Fig. 2b-II.

[g] Fig. 2b-I.

each additional CDCD transfer. However, in the frontal fractionations described here, products were recovered in much more concentrated solutions than in CDCD. For example, trivernolin was obtained in less than a 5-liter frontal fraction, whereas the comparable CDCD

TABLE 2

Comparison of Extraction Methods: Lard Methyl Fsters

Method	Quantity fed		Recovered in effluent		Purity	
	18:0 (g)	18:2 (g)	18:0 (g)	18:2 (g)	18:0 %	18:2 %
Elution[a]	2.5	2.1	2.5	2.1	98	70
Frontal[b]	14.1	11.9	2.3[d]	4.8[e]	91	72
CDCD[c]	34.6	28.9	2.3	7.4	88	70

[a] Calc using Gaussian approx (*1*). 20 g esters placed in Tube 0. V_U = 10 ml, V_L = 39.2 ml.

[b] Experimental results.

[c] Computer simulation. V_U = 10, V_L = 50 ml. Data shown for 550 transfers. Steady state reached at 777 (0.496 g fed per input).

[d] Fig. 3b-I.

[e] Fig. 3b-III.

fraction was dissolved in over 30 liters under the conditions employed in the simulation. Purity of the DV fraction could not be improved in the CDCD fractionation. It is inherent in this approach that if one component of a multicomponent mixture is isolated in high purity in one effluent, the others are concentrated in the other effluent. An exception is the case where one component has a K_D such that it emerges first in the effluent. A small cut of good purity might then be made before the other compounds emerge. The methyl stearate cut from the lard CDCD fractionation could be obtained in this manner. Simulation for a 50-tube apparatus indicated that no stearate would be isolated at high purity.

The recovery of the methyl stearate (Fig. 3b-I) and methyl linoleate (Fig. 3b-III) concentrates indicated that the frontal mode was useful for the isolation of reasonable quantities of relatively minor components of a mixture. This was further demonstrated by the recovery of a small but significant fraction which contained 38% methyl linolenate (Fig. 3b-IV). Since the lard esters contained only 0.9% linolenate, an appreciable concentration was achieved.

These frontal fractionations were designed so that the component having the smallest K_D would just reach a plateau. With certain mixtures it might be advantageous to cut off solute additions before that point. This would shorten the plateaus of the earlier components. For example, a computer simulation showed that if only 65 inputs of lard esters (N_F for methyl oleate) were made, 4.8 g of linoleate (70% purity) could be recovered from the 6.8 g fed. Recovery of stearate would be unaffected except that a greater percentage of that fed would be recovered. These are approximate results, since ideal behavior was assumed in the simulation. The stearate, oleate, and palmitate profiles were described by the frontal equations given earlier. The broad linoleate profile could not be described by either the Gaussian curve or its integral (number inputs $<N_F$).

It was also of interest to try a multiple-input approach to CDCD. Again, computer simulation was employed for the evaluation. One hundred inputs of Vernonia oil, 0.43 g each, were made into Tube 25 of a 100-tube distributor. All of the trivernolin was recovered in lower phase. The divernoloyl and less polar triglyceride fractions were eluted as separate zones in the mobile phase (Fig. 5). Just as with the approach described in the previous paragraph, no simple fundamental mathematical relationship has yet been found to describe these profiles.

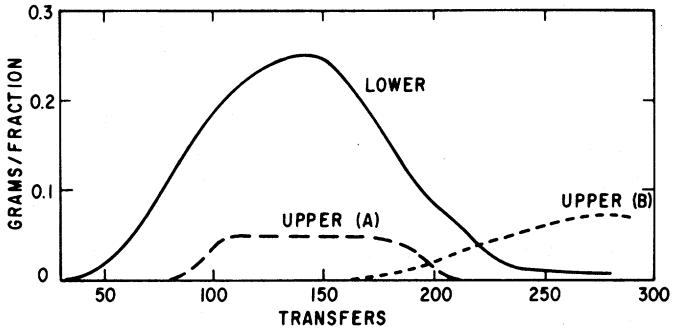


FIG. 5. Multiple input CDCD. $V_U = 15$ ml, $V_L = 50$ ml, $p = 100$, feed tube = 25, 100 inputs—0.43 g Vernonia oil. (—) Trivernolin emerging in acetonitrile-rich phase. Upper (A): Monovernoloyl–diacyl–triglyceride emerging in hexane-rich phase. Upper (B): Divernoloyl–acyl–triglyceride emerging in hexane-rich phase.

CONCLUSION

An equation was derived for determining the number of tubes required for the separation of two solutes by frontal CCD, assuming ideal conditions. This equation, as well as others derived previously, is only an approximation of observed results. The equations allow the experimenter to obtain insights quickly into the nature of the fractionations to be attempted. The two experiments described here demonstrate that the frontal technique is useful for preparative CCD fractionations and for obtaining sizable fractions in which minor components have been concentrated.

Symbols

V_R = retention volume
V_U = volume upper phase
V_L = volume lower phase
K_D = partition coefficient
t = "t value" obtained from probability tables for corresponding value of Y. $t_i = t$ at intersection of two curves.
Y = concn in output/concn in feed. $Y_i = Y$ at intersection of two curves.
V_F = volume of feed solution required to produce frontal output profile
N_F = number of inputs corresponding to V_F
Y_g = [concn in output/concn in feed] × wt % component in mix

p = number of tubes in apparatus
V_T = volume transferred

Double subscripts are used to relate symbols for a particular curve or compound.

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